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A Simple UPLC Analytical Method For Determination Of Antihypertensive Drug Azilsartan In Bulk And Pharmaceutical Dosage Form

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Abstract:
The developed method is very simple and sensitive. The Mobile phase contains 30% Buffer and 70% Acetonitrile. The Buffer was prepared by mixing of 1ml Orthophosphoric acid in 1 litre of water and pH adjusted to 3.0 with Ammonium hydroxide solution. The column used is Acquity Bridge Ethyl siloxane Hybrid Amide having dimension 50mm x2.1mm, 1.7µm. Analysis was carried out at ambient temperature and using 256nm as a detection wavelength. The Mobile phase flow rate was 0.25 ml/min. The developed analytical method was validated as per ICH Q2B guidelines, System suitability of Azilsartan of six replicates injection of standard solution, Reproducibility, Intermediate precision, Recovery, and linearity were fond within limit of acceptance criteria. Linearity was performed 60% to 140% of working concentration. The linearity graph was plotted as peak area verses concentration of Azilsartan in µg/ml. The linearity plot shows the co-relation coefficient of linearity curve is 1.000. The Relative Standard Deviation of area and retention time for system suitability is 0.13%.and 0.79% respectively. The RSD for area in Repeatability is 0.17% and in intermediate sample is 0.12%. The recovery of all three levels with each three different preparation was observed between 98% to 102%. The proposed method had applied to Azilsartan medoxomil drug substance and marketed pharmaceutical finish product.

Key words: *Method development, Validation, Azilsartan modexomil, UPLC.*

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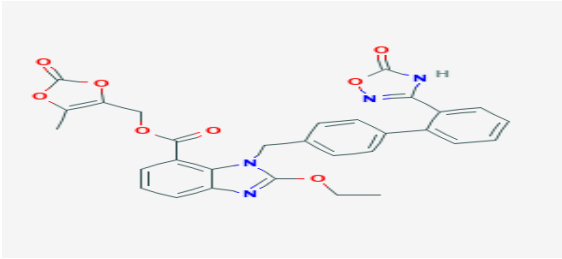
I. Introduction

Azilsartan is Antihypertensive drug substance that used for the treatment of cardiovascular diseases. Marketed pharmaceutical dosage form available in 40mg or 80 mg. It may be used with combination of another drug. This drug belongs to Angiotension II receptor Blocker which lower the blood pressure by improving the blood flow through relaxing the blood vessels and lower the risk of heart strokes. This drug is also known as Edarbi antihypertensive agent. This drug is available in potassium salt form. It is practically insoluble in water and freely soluble in DMF, Methanol, and DMSO.

The IUPAC name: 5-methyl-2-oxo-13-dioxol-4-yl)methyl 2ethoxy-3-[[4-[2-(5-oxo-4H-1,2,4-oxadiazol-3-yl)phenyl]methyl]benzimidazole-4carboxylate

Molecular formula: C30H24N4O8.

The structure of Azilsartan medoxomil:



II. Rresearch Methodology

Instruments and Equipments:

Analysis performed on instrument UPLC WATERS ACQUITY with Empower2 Software and UV–Vis spectrophotometer (Make:LABINDIA) for wavelength maxima determination of Azilsartan modexomil having

concentration of 10.0 ppm. Sartorius Electronic Balance was used for standard and sample weighing, The pH meter (Make : ELICO) was used for pH adjustment for Buffer of mobile phase.

Reagent and Materials:
The reagents and chemical used for Analytical method development are listed below:
Purified Water- UPLC grade, Sd fine-Chem ltd.
Acetonitrile- HPLC Grade Rankem,
Methyl alcohol-A.R Grade Rankem,
Ammonium Hydroxide-A.R Grade Rankem,
OPA- A.R Grade, Sd fine-Chem ltd
DMSO- A.R Grade, Sd fine-Chem ltd
DMF- A.R Grade, Sd fine-Chem ltd

Method development Trials: Based on scanning spectra of Azilsartan Medoxomil standard solution, Wavelength detector was finalized as 256nm as given below. Analytical trials for Method development were taken on Ultra Performance liquid chromatography system using octadecylsilane columns such as Kromasil C18, Acquity C18 BEH (Ethylene Bridged Hybrid using multiple pH trials for phosphate buffer and phosphoric acid as buffer with solvent like Acetonitrile in different ratio.

Determination of wavelength through UV-Spectrophotometer:

The Standard Preparation: Accurately weigh 10.0 milligram of Azilsartan Medoxomil drug substance into 100.00ml. volumetric flask, added about 65 mililitre of Methanol and sonicated to till dissolve, cooled & make up to volume with Methanol. Further, diluted 1.0 ml to a 10.0 ml volumetric flask with Methanol.
This standard solution was scanned in the range of 200 to 400nm in the UV spectrophotometer. The maxima of spectra were 256 nm of Azilsartan Medoxomil solution observed, which will be used in chromatographic condition for method development trials. Picture-1 shows the spectra of Azilsartan medoxomil.

Optimized chromatographic conditions:

Preparation of Buffer solution: 1.0 cc of Orthophosphoric acid diluted in 1000 mililitre of purified water and pH was adjusted to 3.0 using pH meter instrument with Ammonium hydroxide solution.

Preparation of Mobile Phase: Mixed buffer and ACN in the ratio of 30: 70 v/v and degassed in sonicator for 5.0 minutes. Filter mobile phase with 2.0 µm filter using vacuum filtration unit.
Diluent : Mobile phase
Column : ACQUITY BEH AMIDE, 1.7µm, 2.mm X 50mm
Flow rate (ml/min) : 0.25
Detection Wavelength: 256 nm
Column temperature : 25.0 degree Celsius
Injection Volume : 5microlitre
Probable Run time : 3 minutes

Preparation of Standard Solution: Weigh and transfer about 25.0mg of Azilsartan Medoxomil standard into a 50.0ml clean dry volumetric flask, added 30ml of diluent and sonicated till completely dissolve standard and diluted up to the mark. Further diluted 2.0ml of Azilsartan Medoxomil stock solution to a 100.0 milliliter flask with same diluent.

Preparation for drug substance sample: Accurately weigh and transfer 10.0 mg of Azilsartan Medoxomil API into a 10.00ml clean flask, add 7 ml of diluent and sonicate to disperse it completely and diluent added in same flask up to the mark. Shook well and then diluted 1.00 ml of Azilsartan Medoxomil solution to a 100.0cc volumetric flask with diluent.

Preparation of marked drug product: Estimated Avg. weight of marketed finished 20 tablets. All 20 tablets transferred into mortar and crushed with pestle till uniform powder form. Weigh powder equivalent to 50 mg of Azilsartan Medoxomil drug into a 50.0mililitre volumetric flask, added diluent about 35ml in same flask and sonicated this solution to dissolve completely, cool the flask and added diluent up to the mark. Filter the solution with 0.45-micron PVDF syringe filter. Further diluted 1.0ml of filtrate of Azilsartan Medoxomil solution to a 100.0 ml volumetric flask with diluent.

Calculation for marketed drug products:
Assay (mg/tablet) = (SArea x W1 x D(T) x P x AV) / StdArea x D(S) x W2 x 100)

Calculation for drug substance :
Assay (%) = (SArea x W1 x D(T) x P) / StdArea x D(S) x W2)

Where:
SArea = Peak Area response of sample preparation
StdArea = Peak Area response of standard preparation
W1 = Weight of working standard in mg
W2 = Weight of sample in mg
D(S) = Standard solution dilution factor
D(T) = Sample solution dilution factor
P = Purity of standard in %
AV = Tablet Average weight

Result & Discussion: The percentage Assay of Azilsartan Medoxomil of Marketed tablet dosage form was found 100.0%.

III. Analytical method validation:

Method validation of optimized developed method perform as per ICH Guidelines which provide as documented evidence that shows developed method is specific, sensitive, high accuracy, meet the specification results of predetermined values and quality characteristics. The developed analytical method was validated with validation parameters like Specificity, Precision, Linearity, and Accuracy test.

System suitability and Specificity: In this parameter Blank as a diluent and standard solution preparation was injected in the U.P.L.C. system. No peak observed in the blank at the Retention time of analyte. which proved that there is no interference of diluents (Blank) against standard solution as per picture 2 and 3. The interference was also confirmed with a degradation study using the different stress conditions. six replicate injection of standard solution injected into the chromatograph to confirm the system suitability criteria. Blank, Standard and sample chromatogram shown in Picture 2,3, and 4. And System Suitability results were shown in Table-1 The Percentage RSD of retention time and area response of six replicate injection of Azilsartan is 0.79 and 0.13.

Precision: In Precision confirms the closeness of observed results of the sample prepared from same uniform parent sample under specified chromatographic conditions. It is two types. 1) Repeatability precision, and 2) Intermediate precision.

Repeatability: In this chapter all 6- sample solutions are prepared from homogeneous mixed samples. It is injected in Special ultra performance technique of liquid chromatography system. The Relative Standard Deviation of R.T. and Area response of eluted peak for the Six sample injections was observed within the specified limits. The results were tabulated in Table-2. The R.S.D. for the retention time and area response of repeatability samples injected into the system was 1.0% and 0.17%.

Intermediate Precision: Intermediate precision sample was prepared by different analyst on next day. Which confirm the Ruggedness of the analytical technique. The six samples were prepared from same parent homogeneous samples injected into the Ultra Performance of fluid flow Liquid Chromatography system. The Relative standard deviation of RT and Area of Six injected sample was found within the acceptance limit. The R.S.D. for the eluted time and area of injected samples is 0.80 and 0.12 respectively. The results were shown in table 3.

Linearity: It is an Analytical procedure technique which shows the peak absorbance results varies against vary in the concentration of the analyte up to the specified limit range.

Preparation of Linearity stock solution:

Accurately weight and transferred 10.0 mg of standards into a 10.0ml volumetric flask, added 6.0 ml diluent and sonicated to dissolve it completely and the flask is diluted upto the mark with the diluent. **(Stock solution)**

Linearity solution Preparation:

Linearity Level – 1 (60%):

Diluted 0.06CC of stock solution with diluent into 10.0 ml flask.

Linearity Level – 2 (80%):

0.080 ml of stock solution diluted to10.0cc of volumetric flask with diluent.

Linearity Level – 3 (100%):

0.100 mililitre of Linearity stock solution transfers into 10.00 ml of volumetric flask and dilute up to the mark with diluents.

Linearity Level – 4 (120%):

0.120 ml of stock solution of linearity diluted into 10ml of flask.

Linearity Level – 5 (140%):

0.140 cc of stock solution prepared for linearity was transferred into 10mililitre of volumetric flask. The diluent is added into the flask up to the mark.

Procedure: Each level injected into the UPLC chromatographic system and measured the covered area response. Plot a graph of peak covered area response versus actual concentration observed. In the linearity plotted graph X-axis was concentration and on Y-axis was Peak covered area response and correlation coefficient was calculated. The Linearity graph plot peak area verses concentration shown in Picture-5 and Table-7. The observed co-relation coefficient of linearity levels from 6.06ppm to 14.14 ppm is 1.000.

LOD and LOQ:

LOD: It is lowest concentration of the analytical method in which analyte is detected but not necessary to be quantify.

LOQ: It is lowest concentration of the analytical method in which analyte to be quantify.

LOD= 3.3 × σ/S

LOQ = 10×σ/S

Where

σ = Standard deviation of the response

S = Slope of the calibration curve

Observation: On the evaluation of above results the LOD and LOQ for the Azilsartan Medoxomil was found to be 0.5853 µg/ml and 1.7738µg/ml respectively.

Accuracy: It is an analytical procedure parameter which represents the nearness of series of different levels observed results closeness to true theoretical results. Sometimes it is defined as trueness.

Accuracy solution preparations:

Accuracy standard solution: Accurately transferred 20miligram of Azilsartan Medoxomil into a 20ml clean dry volumetric flask, added 17 ml diluent and sonicated to dissolve, cooled and make volume up to the mark with the diluent. Further diluted 1ml to 100ml with diluent.

Accuracy sample preparation:

Level-1 (50 % working standard): Accurately weigh and transfer 5.0 mg of Azilsartan Medoxomil is taken into a 10ml clean dry volumetric flask, add Diluent and sonicate to dissolve it completely and make volume up to the mark with the diluent. Further pipette 0.1ml of Azilsartan Medoxomil of the above stock solution into a 10ml volumetric flask and diluent added up to the mark. Prepared in triplicates. Results tabulated in Table-4.

Level-2 (100 % working standard) Accurately transfer 25.0 mg of Azilsartan Medoxomil is taken into a 25.0 ml volumetric flask, added diluent and sonicate till dissolve and make volume up to the mark with the diluent. Further pipette 0.10ml of Azilsartan Medoxomil of the above stock solution into a 10.0ml clean volumetric flask and diluted up to the mark with diluent. 3 preparations done. Table-5 shows the results of Accuracy Level-2.

Level-3 (150 % working standard): weigh exactly and transfer 15.0 mg of Azilsartan Medoxomil is taken into a 10ml clean volumetric flask, added diluent and make volume up to the mark with the diluent. Further diluted 0.1ml of the above stock solution into a 10ml volumetric flask and dilute up to the mark with diluent. Three preparations were made. Accuracy Level-3 results are in Table-6

IV. DEGRADATION STUDIES:

The International Conference on Harmonization (ICH) guideline entitled stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. The aim of this work was to perform the stress degradation studies on the Azilsartan Medoxomil using the proposed method.

Preparation of stock:

Crushed 10 tablets in mortar and pestle, then mixed powder uniformly and transfer equivalent to 10 mg of Azilsartan Medoxomil sample into a 10mL clean dry volumetric flask added about 6.5 mL of diluent and sonicate it up to 30 mins to dissolve it completely and make volume up to the mark with the same solvent. Then it is filtered through 0.45µ syringe filter. (Stock solution).

Basic Hydrolysis:

Pipette 0.50ml of above filtered stock solution into a 50ml volumetric flask and added 5ml of 0.1N NaOH solution in same volumetric flask. The flask was kept at 60ºC for 24hours, cool and then neutralized with 5.0 ml of 0.1N HCl and make volume up to the mark with diluent.

Acid Hydrolysis:

Add 0.5ml of above solution into a 50ml volumetric flask Than added 1ml of 0.1N HCl in same flask. Mixed solution thoroughly and the volumetric flask was kept at 60ºC for 24 hours, cool and then neutralized with 1ml of 0.1 N NaOH and make up to the mark with diluent.

Oxidation Hydrolysis:

0.50ml of stock solution took into a 50ml volumetric flask and adding 5.0ml of 10% w/v of hydrogen peroxide solution in the same volumetric flask. The flask was then kept at room temperature for 30 minutes and the volume was made up to the mark with the same diluent.

Thermal degradation:

The powder of Azilsartan Medoxomil sample was taken in Petri dish and kept in oven at 80 ºC for 3 hours. Then the sample solution was prepared from exposed powder sample with known concentration 10 µg/ml in diluent, then filter the solution with 0.45 µ syringe filters and injected into UPLC.

Photo degradation:

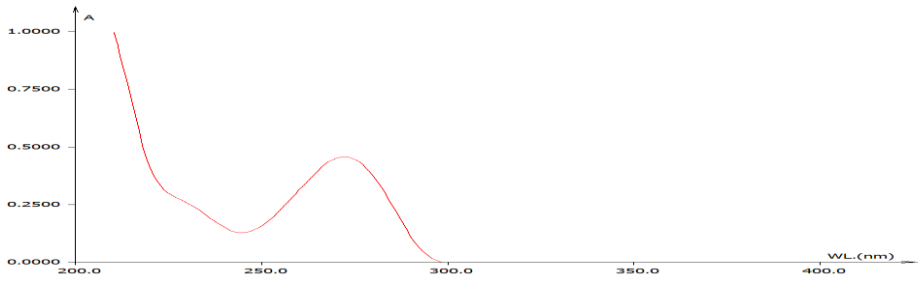
The prepared stock solution taken in 10ml volumetric flask and was exposed to sunlight for 24hrs and the volume was made up to the mark with diluent.

V. CONCLUSION:

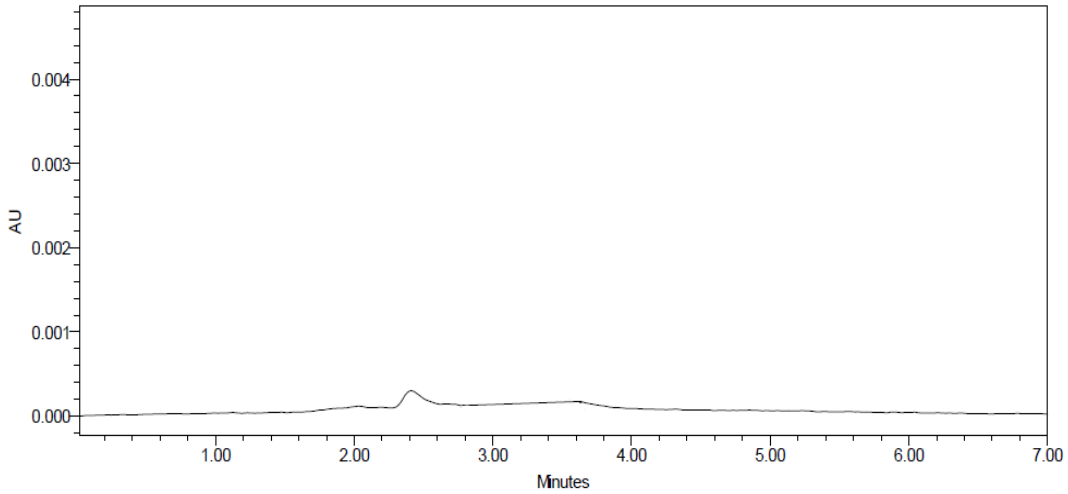
The Azilsartan peak is eluted within 1minute, Hence the developed method is time saving analytical method. The Mobile phase preparation is very simple. Based on observation of table 8, it was observed that the solution of Azilsartan Medoxomil tablets dosage form shows degradation in basic and Acidic stress condition about 3-4 %. In oxidation stress condition Azilsartan degraded about 4%, Hence it is highly unstable in oxidation condition and also it is thermally unstable. It was degraded about 4% in thermal stress condition. So Azilsartan Medoxomil is very sensitive in Acidic, Basic, oxidation and thermally hot environment condition. Hence the method is specific. The % RSD of all six-sample preparation of the repeatability and intermediate precision is within limit. So the developed method is precise.

The observed Correlation coefficient of linearity plot of Azilsartan Area response against concentration of level is 0.999 which is within limit, hence method is Linear in concentration range of 6ppm to 14 ppm of Azilsartan. The % recovery of mean of all levels is between 98% to 102%. Hence the method is accurate.

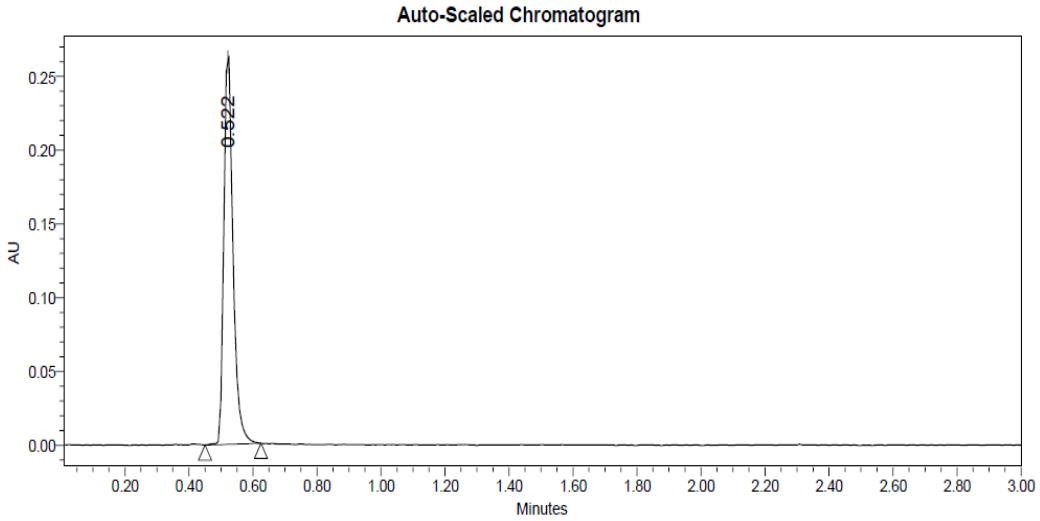
VI. FIGURES AND TABLES:



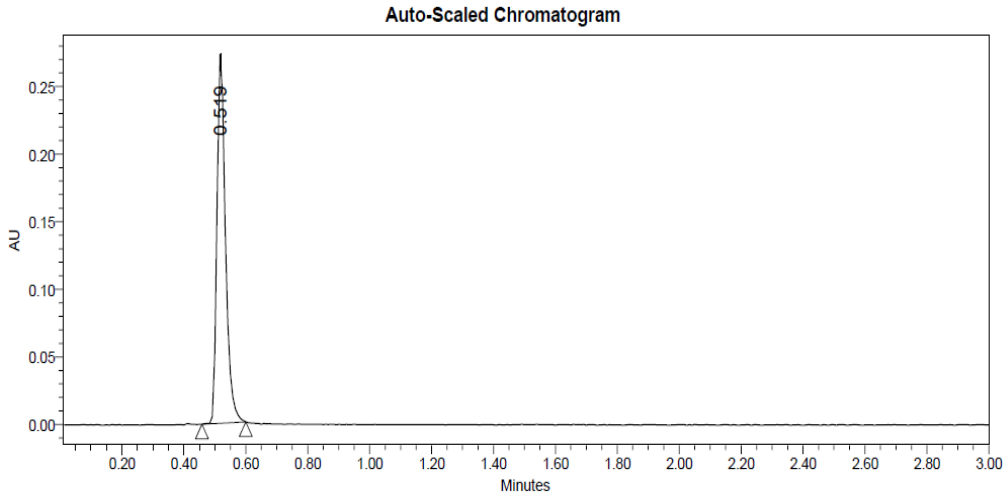
Picture-1: UV Spectrum of Azilsartan Medoxomil



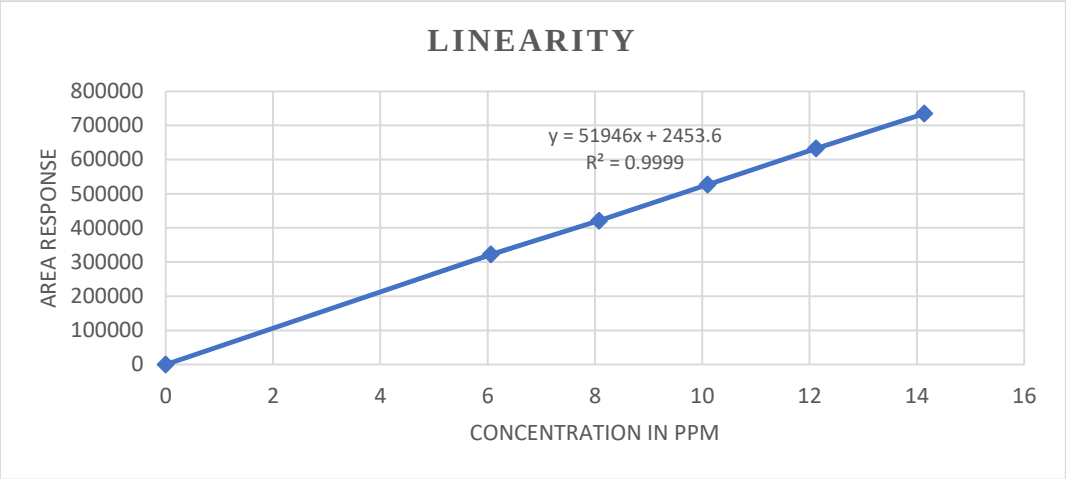
Picture-2: Blank Injection Chromatogram



Picture-3: Standard Injection Chromatogram



Picture-4: Sample Injection Chromatogram



Picture-5-: Linearity Level plot (Area response against concentration in ppm)

Table-1: System Suitability results for Azilsartan Medoxomil

Sr. No.	Peak Name	Retention time	Area (µV*sec)
1	Azilsartan	0.52	515856
2	Azilsartan	0.52	516864
3	Azilsartan	0.52	515752
4	Azilsartan	0.52	514886
5	Azilsartan	0.52	515674
6	Azilsartan	0.51	515423
	Mean	0.52	515743
	Std. Dev	0.004	649.785
	%RSD	0.79	0.13

Table-2: Repeatability Results for Azilsartan Medoxomil

Inj. No.	Peak Name	Retention time	Area (µV*sec)
1	Azilsartan	0.52	515354
2	Azilsartan	0.52	514767
3	Azilsartan	0.52	515643
4	Azilsartan	0.51	516895
5	Azilsartan	0.52	514356
6	Azilsartan	0.51	515754
	Mean	**0.517**	**515462**
	Std. Dev	**0.0052**	**881.9793**
	%RSD	**1.00**	**0.17**

Table-3: Intermediate precision Results for Azilsartan Medoxomil

Sr.No.	Peak Name	RT	Area (µV*sec)
1	Azilsartan	0.51	517659
2	Azilsartan	0.52	518596
3	Azilsartan	0.51	518632
4	Azilsartan	0.51	519648
5	Azilsartan	0.51	518865
6	Azilsartan	0.51	518823
	Mean	**0.512**	**518704**

	Std. Dev	**0.004**	**638.763**
	%RSD	**0.80**	**0.12**

Table-4 Accuracy Results for concentration-50%

Sr.No.	Name	Area (Y)	Amount Added (ppm)	Amount Found (ppm)	% Recovery
1	Azilsartan	264379	5	5.04	100.84
2	Azilsartan	263287	5	5.02	100.42
3	Azilsartan	259799	5	4.95	99.08
	Mean=	262488.3	5	5.01	100.12

Table-5: Accuracy Results for concentration-100%

Sr.No.	Peak	Area (Y)	Amount Added (ppm)	Amount Found (ppm)	% Recovery
1	Azilsartan	530104	10	10.16	101.58
2	Azilsartan	519898	10	9.96	99.61
3	Azilsartan	526232	10	10.08	100.83
	Mean=	525411.3	10	10.07	100.67

Table-6: Accuracy Results for concentration-150%

Sr.No.	Peak Name	Area (Y)	Amount Added (ppm)	Amount Found (ppm)	% Recovery
1	Azilsartan	787574	15	15.11	100.76
2	Azilsartan	784512	15	15.06	100.37
3	Azilsartan	788357	15	15.13	100.86
	Mean=	786814.3	15	15.10	100.66

Table-7: Azilsartan Medoxomil Linearity results

Linearity Level	Concentration(ppm)	Area
1	0	0
2	6.06	322236
3	8.08	421132
4	10.1	526989
5	12.12	632655
Correlation Coefficient		1.000

Table-8: Results of Degradation Studies of Azilsartan Medoxomil

Stress Condition	Azilsartan Medoxomil	
	Sample Area	% Degradation
Control	518839	0
Acid Hydrolysis	501853	3.274
Basic Hydrolysis	499776	3.674
Oxidation	499354	3.756
Thermal degradation	499998	3.631
Photo degradation	515280	0.686

REFERENCES

[1]. Azilsartan: Side Effects, Dosage & Uses - Drugs.Com
[2]. ICH Q2b: Validation Of Analytical Procedure; Methodology (International Conferences On Harmonization Of Technical Requirements For The Registration Of Drugs For Human Use, Geneva, Switzerland, Nov 2003).
[3]. Skoog D.A., Holler F.J., Nieman D.A.," Principle Of Instrumental Analysis", 6th Ed Reprint, Thomson Brooks/Cole Publication, 2004 ; 300-351.(UV).
[4]. Azilsartan Medoxomil | C30H24N4O8 - Pubchem (Nih.Gov)
[5]. Fronk A.S.,"Handbook Of Instrumental Techniques For Analytical Chemistry", 1st Edn. Pearson Education, 2004; 7.
[6]. Jeet P. Shah1, Dr. Ashok H. Akabari2, Development And Validation Of Analytical Method For Estimation Of Azilsartan Medoxomil And Chlorthalidone In API And Pharmaceutical Dosage Form, International Journal Of Pharmaceutical Research And Applications, Volume 7, Issue 3 May-June 2022, Pp: 1251-1262.